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EPN (O-Ethyl-O-p-nitrophenyl phenylphosphonothioate): Position Document 1

(U.S.) Environmental Protection Agency
Arlington, VA

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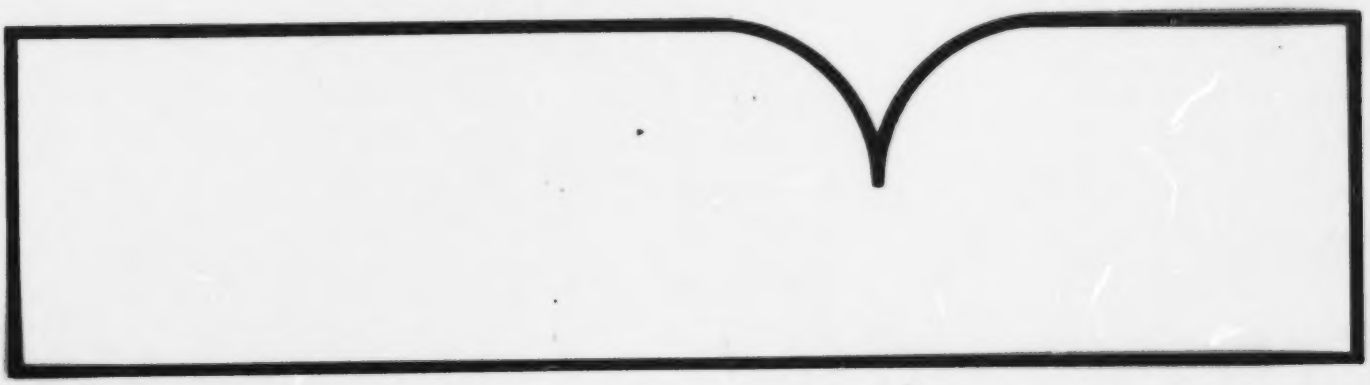

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EPN: Position Document 1

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U.S. Environmental Protection Agency
Project Manager: Patrick Miller

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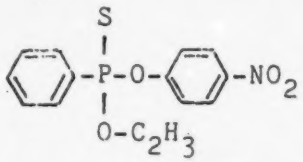
EPN: Position Document 1

I. BACKGROUND

A. Chemical and Physical Data

EPN (O-Ethyl-O-p-nitrophenyl phenylphosphonothioate) is a non-halogenated, aromatic, phosphonothioate organophosphorus compound. It has the empirical formula $C_{14}H_{14}NO_4PS$. The structural formula is:



Other chemical names used by manufacturers are ethyl p-nitrophenyl thionobenzene phosphonate and O-Ethyl-O-p-nitrophenyl benzene thiophosphonate.

The pure compound occurs as a light-yellow, crystalline powder with an aromatic odor, and the technical grade is a reddish-yellow, oily liquid (Nissan 1976). The chemical has a molecular weight of 323.3. EPN is only slightly soluble in water and is miscible with benzene, toluene, xylene, acetone, isopropyl alcohol, and methanol (Meister 1977). The partition coefficient in octanol/water is 48,253 (Shafik et al. 1976). EPN has a melting point of 34.5°C, vapor pressure of 0.03 mmHg at 100°C, and specific gravity of 1.27 at 20°C (Nissan 1976). The hydrolytic half-life of EPN is 40.9 hours at a pH of 6 and temperature of 72°C ± 2°C (Shafik et al. 1976).

Colorimetric and gas chromatographic methods of analysis for quantitatively determining EPN residues in and on plant species have been devised by Averell and Norris (1948), Coffin and McKinley (1963), Kirkland and Pease (1967), and Laski (1974). Bhagwat and Ramachandran (1974) described a simple and rapid spectrophotometric method for determination of EPN, EPNO, and p-nitrophenol in aqueous suspensions and enzymatic digests. Other methods of analysis are cited in Kirkland and Pease (1967).

B. Formulation and Class

EPN is classed and used as a non-systemic insecticide-acaricide and is available in emulsifiable concentrates, dusts, wettable powders, and granular formulations. The standard commercial formulation is an emulsifiable concentrate alone or in combination with another pesticide. The concentration of EPN ranges from 21 to 55% in these various formulations. EPN is registered in combination with methyl parathion, guthion, toxaphene, and parathion.

C. Registered Uses and Production

EPN was patented in 1950 by E.I. du Pont de Ne Mours, Inc. (Patent Number 2503390), and the first tolerances were issued for the chemical in the same year. Twenty-six companies hold Federal registrations and formulate 72

registered products. Six companies have former state registrations[1/] and formulate 10 products.

Environmental Protection Agency (EPA) records indicate that a total of 4,126,500 pounds of EPN were used in the United States during 1974 (EPA 1977). Of this total, about 2 pounds were used in industry; 8,000 pounds were used in government; and 4,118,500 pounds were used in agriculture. Of the agriculture total, 4,500 pounds were used on beans; 326,000 pounds on corn; and 3,788,000 pounds on cotton. Several reports indicate that the amount of EPN used in 1974 probably is much less than the amount currently used. In 1976, EPN usage in Mississippi alone amounted to 2,469,700 pounds (MSCL 1977). And in California in 1977, EPN usage increased from a 7-year average (1970 to 1976) of 9,600 pounds to a total of 293,500 pounds. Of this 1977 total, 289,000 pounds were used on cotton (Memo 1978d).

This chemical is registered for ground and aerial application as a mosquito larvicide by Mosquito Abatement Districts, Public Health Officials, and other trained personnel of Public Mosquito Control Programs (Memo 1978a). All tolerance petitions have been submitted by E.I. du Pont de Ne Mours Co., Inc.

1/ Pesticide products formerly registered under state pesticide registration laws and shipped or distributed for sale solely within intrastate commerce are subject to Federal pesticide regulations under 40 CFR Section 162.17(a). Application has been made to obtain Federal registration for intrastate use of these products.

Table 1. Application of EPN to Cropland Sites[a/] During FY 1969 to 1974

FY	Frequency of Application	Avg Rate of Application
1969	0.1% (2 sites out of 1,684)	1.5 pounds/- acre on cotton
1970	0.2% (3 sites out of 1,346)	1.1 pounds/- acre on cotton
1972	0% (0 sites out of 1,473)	
1973	0.1% (1 site out of 1,402)	3.0 pounds/- acre on cotton
1974	0.1% (1 site out of 1,165)	0.38 pounds/- acre on sweet corn

a/ Location of sites were not listed in the Monitoring Branch's report.

The National Soil Monitoring Program, conducted by EPA's Ecological Monitoring Branch, has collected information on the application of EPN by landowners (Memo 1977). Most recorded applications were on cotton. The data in Table 1 indicates the percentage of sample cropland sites to which EPN was applied. The percentage of application was low (<1%) and remained constant between fiscal year (FY) 1969 and FY 1974.

The use data available to the Agency generally indicates that applicators are most at risk from exposure to EPN. The general population would be at risk from EPN application near homes and other work or recreational areas, or from EPN residues on food (mostly cottonseed meal and oil) [see also Section III.A.(3)].

D. Tolerances

Established residue tolerances for EPN in or on raw agricultural commodities are listed in 40 CFR 180.119 as follow: 3 parts per million (ppm) in or on apples, apricots, beans, beets (with or without tops) or beet greens alone, blackberries, boysenberries, cherries, citrus fruits, corn, dewberries, grapes, lettuce, loganberries, nectarines, olives, peaches, pears, pineapples, plums (fresh prunes), quinces, raspberries, rutabagas (with or without tops) or rutabaga tops, spinach, strawberries, sugar beets (but not sugar beet tops), tomatoes, turnips (with or without tops) or turnip greens, and youngberries; 0.5 ppm in or on almonds, cottonseed, pecans, and walnuts; and 0.05 ppm (negligible residue) in or on soybeans.

E. Metabolism

(1) Mammals

Neal and DuBois (1965) first proposed a metabolic pathway for biotransformation of EPN to p-nitrophenol in the mammalian system which involved oxidative desulfuration to form the active toxic metabolite EPNO (O-ethyl O-p-nitrophenyl phenyl phosphonate) and subsequent hydrolysis by esterases.

Based on the works of Ahmed et al. (1958) and Nakatsugawa et al. (1968), Menn (1971) proposed a metabolic pathway for EPN in animals consisting of the toxic metabolite EPNO, and detoxification products EPPTA [O-ethyl phenyl

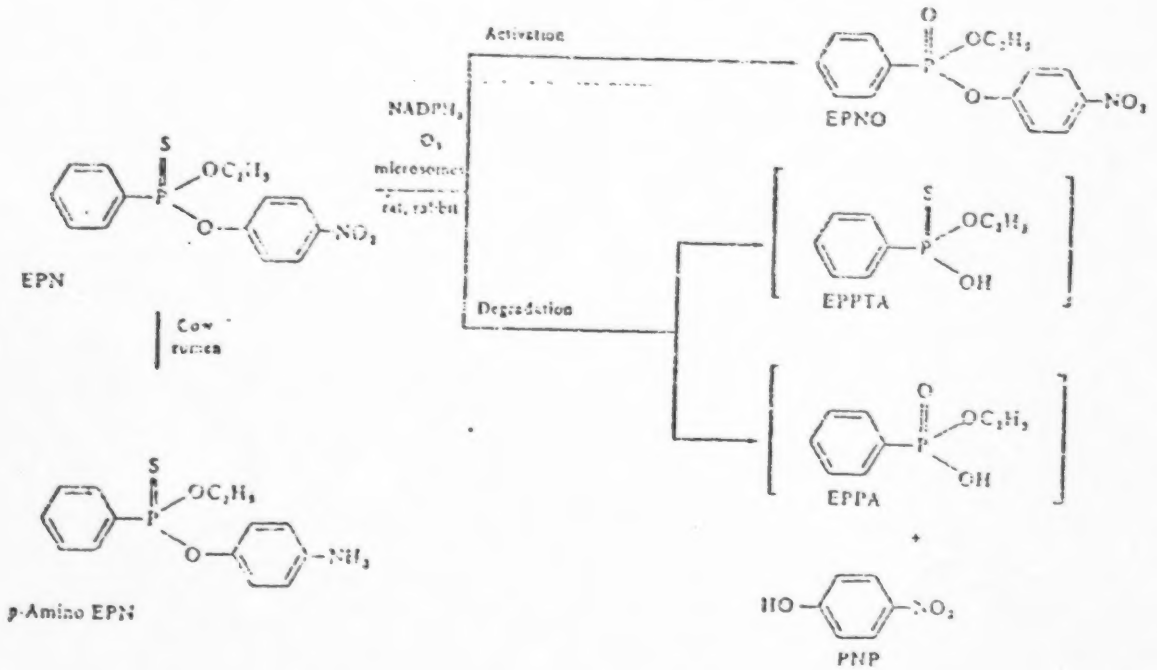


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Figure 1. Proposed Metabolic Pathway for EPN in the Mammalian System. Reprinted, by permission, from J.J. Menn, Terminal residues of phosphonate insecticides, Page 65, IUPAC, Pesticide terminal residues (International Symposium: Tel Aviv, 1971). © 1971 by International Union of Pure and Applied Chemistry.

phosphonothioic acid], EPPA [O-ethyl phenyl phosphonic acid], PNP [p-nitrophenol], and amino-EPN (see Figure 1).

DuPont (1977a)[2/] reported small amounts of radioactivity in the gastrointestinal tract (0.4%), carcass (1.1%), hide (5.6%), and internal organs (0.2%) of a single male rat orally dosed with 5.5 mg of ^{14}C-(phenylphosphonothioate) EPN after three days. The high percentage of radioactivity in the hide was attributed to grooming. Excretory products accounted for the major radioactivity recovered. Within 72 hours, 58% of the original radioactivity was in the rat urine and 26% was in the feces. No respired ^{14}C-EPN was detected. The major metabolites in both urine and feces were EPPTA, EPPA, phenylphosphonothioic acid, and phenylphosphonic acid. No intact EPN or EPNO was found.

Ahmed et al. (1958), using column chromatography and infrared spectra methods, analyzed EPN degradation products in one-liter samples of cow rumen juice to which 300 ppm each of EPN and its oxygen analogue, EPNO, had been added. After three hours, 5% of the EPN was recovered as amino-EPN and 50% of the EPNO as amino-EPNO. The remaining compounds were considered as products of hydrolysis.

(2) Invertebrates

Carlson (1972) examined the ability of the quahaug, a mollusc (Mercenaria mercenaria), to oxidize or reduce EPN

2/ Studies submitted by registrants in support of tolerance petitions are automatically classified CONFIDENTIAL prior to action on the petition.

by analyzing various tissues for the presence of p-nitrophenol. This byproduct was not detected in whole liver homogenates, in 9,000 gram supernatent fractions or microsomal fractions of the hepatopancreas, or in whole homogenates of mantle, foot, and gills. The author concluded that the quahaug was unable to metabolize the EPN. This finding is consistent with data showing that EPN is apparently more toxic to aquatic invertebrates than to aquatic vertebrates [see Section III.B].

(3) Plants

DuPont (1977a) reported rapid metabolic degradation of ^{14}C-(phenylphosphonothioate) EPN on young greenhouse cotton plants sprayed once with the equivalent of 8 ounces EPN/100 gal water. EPPA was the primary metabolite found. After two weeks, 43% of the total radioactivity consisted of EPPA, 23% consisted of intact EPN, 20% consisted of phenylphosphonic acid, and 14% consisted of unextracted residue and unknown polar and non-polar compounds.

(4) Model Ecosystem Study

Algae (Ocdogonium), daphnia (Daphnia), snail (Physa), mosquito (Culex), and fish (Gambusia) which had been placed in a model ecosystem were analyzed for biotransformation of ^{14}C-(phenylphosphonothioate) by Metcalf (Letter 1977a).[3/] This system consisted of a glass aquarium contain-

3/ The author is preparing this material for publication and has asked that it not be released.

ing a sloping shelf of 15 kg washed white quartz sand in addition to the organisms. One mg, or the equivalent of 0.2 kg/hectare (0.18 pounds/acre), of ^{14}C-EPN was applied to the leaves of sorghum plants. Thirty-three days after application the organisms were removed and analyzed using thin-layer chromatography and radiochemistry. All the organisms except the snail appeared to metabolize EPN and contained various metabolic products of EPN including amino-EPN, EPNO, phenylphosphonic acid, EPPA, and EPPTA.

F. Environmental Fate

(1) Persistance: Soils

Terriere and Ingalsbe (1953), using a mosquito larvae bioassay technique, analyzed for EPN deposits in the upper six inches of a sandy loam soil treated with 10 pounds of active ingredient per acre. Residues of 0.2 ppm EPN were found to persist in the soil after two years.

Metcalf (Letter 1977a) studied the persistence of ^{14}C EPN and ^{14}C parathion in the sand of a model ecosystem (see Section I.E.(4) for protocol). EPN was found to be nearly three times as persistent as parathion, comprising 84.5% of the total extracted ^{14}C from sand after 33 days. The remaining extracted ^{14}C products were amino-EPN$_2$ (0.4%), EPNO (0.57%), polar compounds (4.66%), and unknowns (9.88%).

DuPont (1977a) reported that the half-life of intact ^{14}C EPN in soil ranged from two weeks in Dundee silt

loam to one month in Keyport silt loam under actual field conditions in soil treated with 1.8 pounds active ingredient per acre. After four months more than 90% of the residual radioactivity was in the upper 0- to 3-inch layer in both types of soil. The only metabolites found were those possessing the phenylphosphonic acid moiety. In greenhouse soil studies, the half-life of intact EPN on Fallsington sandy loam was reported to be five to six weeks.

(2) Persistence: Water

According to the findings of Shafik et al. (1976), EPN hydrolyzes less slowly than other organophosphate compounds. It has a hydrolytic half-life of 40.9 hours at a pH of 6 and temperature of 72°C ± 2°C. Acute toxicity data available to the Agency show that 96-hr LC_{50}s for aquatic vertebrates range from 16.5 to 80 ppb [see Section III.B]. EPN appears to be more toxic to aquatic invertebrates.

(3) Bioaccumulation

EPN bioaccumulates in certain organisms. Metcalf (Letter 1977a), in a model ecosystem study previously described in Section I.E.(4), showed that EPN accumulates in organisms to a higher degree than parathion, but accumulates less than leptophos and desbromoleptophos. EPN ecological magnification[4/] values were 73 for alga, 77 for daphnia,

4/ "Ecological magnification" and "Bioconcentration" are here defined as the concentration of the parent compound in the organism as compared to concentration in water.

12,561 for snails, 315 for mosquitoes, and 346 for fish (Gambusia).

Schimmel et al. (1978), in 96-hr toxicity tests, reported an average bioconcentration factor of 260 in spot (Leiostoma xanthurus). In another 96-hr toxicity test, Schimmel (Letter 1978a) reported an average bioconcentration factor of 756 for pinfish (Lagodon rhomboides).

In an EPN uptake/depuration study, Schimmel (Letter 1978b) reported a bioconcentration factor of approximately 700X in pinfish. EPN was readily concentrated in the tissues of the fish to an equilibrium concentration of approximately 1.7 ug/g (1.7 ppm). Within one week after termination of the exposure, no EPN was detected in the tissues.

(4) Transport

The findings of DuPont (1977a), previously cited in Section F.(1), show that there is little movement of EPN in soil.

G. Residues

(1) Air, Water, and Soil

Neither the EPA National Air Monitoring Program, (Memo 1977) nor the EPA National Estuarine Monitoring Program has data on EPN.

EPA's National Soil Monitoring Program has sampled agricultural soils to assess pesticide residue concentrations (Memo 1978b). EPN was not detected during the widespread

agricultural monitoring from FY 1969 to 1973. However, detectable concentrations of EPN were found in 3 of 30 samples collected from cotton fields in Mississippi in 1976. Comparison of the 1976 results with the results obtained in 1972 indicates that residual EPN in soil may be increasing.

(2) Feed

Osburn et al. (1960) studied the persistence of EPN on forage under sprayed pecan trees in Albany, Georgia, during 1957 and 1958. EPN was applied three times at two-week intervals at a rate of 2 pounds of 25% wettable powder in 100 gal water. Residues were analyzed using the method of Averell and Norris (1948). The following results were obtained (Table 2). Over 90% of the initial residues were lost after two weeks in all but one trial. There is no established tolerance level for residues of EPN on grass or grass hay.

Table 2. EPN Residues on Forage Under Sprayed Pecan Trees During 1957 and 1958

Days Following Treatment	Residues (ppm)					
	First Application		Second Application		Third Application	
	1957	1958	1957	1958	1957	1958
0	36.4	76.4	33.2	39.0	24.5	33.2
7	4.0	4.9	6.4	7.9	0.6	8.8
13	0.9	3.5	2.0	2.5	0.4	5.8

(3) Food: FDA Commodity Survey

The Food and Drug Administration (FDA) has been collecting food and feed samples on domestic and imported

products for a number of years, analyzing each sample to determine pesticide residue levels and frequency of occurrence (FDA undated). The FDA surveillance program[5/] surveyed for EPN during FY 1972 through 1976. Residues of EPN were found only in 1975 on the following domestic products (Table 3).

Table 3. EPN Residues Reported by FDA Surveillance Program for FY 1972 to 1976

FY	State	Commodity	Residue (ppm)
1975	NY	large fruits (baby food)	trace
1975	MA	Infant Jr. Foods (apples)	trace
1975	FL	nuts (peanuts)[a/]	0.18
1975	FL	nuts (peanuts)[a/]	0.20

a/ There is no established tolerance level for EPN on peanuts.

A review of the Pesticides Monitoring Journal for the years 1964 through 1974 revealed no other positive or negative reports for EPN residues on food and feed products.

(4) Plants

Wolfenbarger et al. (1970), using gas liquid chromatography (GLC), analyzed surface and internal residues following foliar treatment of conventional and ultra low volume (ULV) emulsion sprays of 1.12 kg EPN/ha (1 pound EPN/acre) to cotton foliage in Brownsville, Texas. The original external residue concentration declined by 50% 15.1 and 19.7 hours

5/ The FDA Program under which continuous monitoring is carried out.

after treatment with the ULV and the conventional spray, respectively. After three days, external residues on conventional and ULV-treated cotton plants were 0.019 and 0.01 ug/cm^2, respectively. No residues were detected on internal samples.

(5) Animals

Hodge et al. (1954) analyzed tissues from rats used in a two-year dietary study[6/] and found that there was little tendency for storage of EPN (Table 4).

Table 4. Residues Found in the Tissues of Rats After a Two-Year Feeding Study

		Ranges in Tissue Residues (ppm)				
Sex	Diets (ppm)	Liver	Kidney	Perirenal fat	Brain	Spleen
M	50 & 150	0-5	0-2	0-2	0-7	0-6
	450	4-7	2-3	2-4	4-6	5-9
F	25 & 75	0-9	0-3	0-4	0-6	0-22
	225	5-7	2-5	3	3-12	5-31

(6) Humans

The National Human Monitoring Program for Pesticides, through its cooperative arrangement with the National Center for Health Statistics of the U.S. Public Health Service, is sampling human urine for p-nitrophenol (Memo 1977). This survey, however, will not be completed until 1979. Preliminary data suggest that human exposure to parathion, methyl parathion, and EPN (all of which produce p-nitrophenol upon metabolism) is at a low frequency

6/ It is not known if the tissue samples were analyzed immediately after termination of the experiment, or if there was a time lapse.

and level. To date approximately 400 out of a total of 7,500 samples taken of the general population have been analyzed. p-Nitrophenol has been detected in approximately 2% of the samples.

H. Toxicity Studies

(1) Aquatic Species

A summary of the available data on the acute toxicity of EPN to fresh water, marine, and estuarine species is listed in Table 5.

(2) Birds

A sumary of the available data concerning the toxicity of EPN to birds is listed in Table 6.

(3) Mammalian Species

A summary of the available data concerning the toxicity of EPN to mammals is listed in Table 7. Additional information is provided by Hodge et al. (1954), Frawley et al. (1957), and Radeleff and Woodard (1957).

Palmer (1974), in acute oral toxicity studies, found minimal toxic doses of 2.5 mg/kg for calves and 25 mg/kg for yearling cattle and sheep. Maximal non-toxic doses were 1 mg/kg for calves and 10 mg/kg for yearling cattle and sheep.

Palmer (1974) estimated that unrestricted grazing by livestock of vegetation treated at recommended application rates could potentially expose these animals to doses of 5 to 84 mg EPN/kg body weight per day. An application rate of

Table 5. Acute Toxicity of EPN to Fresh Water and Marine/Estuarine Species

Species	Exposure Time/-Toxicity Parameter	Toxicity Value (ppb)	Reference
juvenile striped bass (Morone Saxatilis)	96 hr/TLm[a/]	60	Korn and Earnest (1974)
rice field fish (Aplocheilus latipes)	24 hr/LC-50[b/]	290	Shim and Self (1973)
fathead minnow (Pimephales promelas)	96 hr/TLm	1,000	Henderson and Pickering (1957)
spot (Leistomus xanthurus)	96 hr/LC-50	25.6	Schimmel et al. (1978)
bluegill sunfish (Lepomis macrochirus)	96 hr/LC-50	80	Letter (1977b)[d/]
rainbow trout (Salmo gairdnerii)	96 hr/LC-50	190	Letter (1977b)
carp (Cyprinus carpio)	48 hr/TLm	350	Nishiuchi and Hashimoto (1967)
carp (Cyprinus auratus)	48 hr/TLm	320	Nishiuchi and Hashimoto (1967)
scud (Gammarus lacustris)	96 hr/LC-50	15	Sanders (1969)
daphnia (Daphnia pulex)	3 hr/TLm	1.2	Nishiuchi and Hashimoto (1967)
daphnia (Moina macrocopa)	3 hr/TLm	7.1	Nishiuchi and Hashimoto (1967)
daphnia (Daphnia magna)	48 hr/LC-50	0.32	Letter (1977b)
pink shrimp (Penaeus duorarum)	96 hr/LC-50[c/]	0.29	Schimmel et al. (1978)
pinfish (Lagodon rhomboides)	96 hr/LC-50	16.4	Letter (1978)

a/ Median tolerance limit.
b/ Lethal concentration to 50% of the population.
c/ EPN is extremely toxic to pink shrimp (Penaeus duorarum). Schimmel et al. (1977) reported that at a concentration of EPN below the limit of detectability (0.02 ppb), 20% of the shrimp used in his experiment died.
d/ Studies submitted by registrants in support of tolerance petitions are classified CONFIDENTIAL and may not be released without permission.

Table 6. Acute Toxicity of EPN to Bird Species

Species	Toxicity Parameter	Toxicity Value	Reference
bobwhite (*Colinus virginianus*)	LC-50[a/]	349 ppm	Hill et al. (1975
Japanese quail (*Coturnix coturnix japonica*)	LC-50[a/]	443 ppm	Hill et al. (1975)
	Oral LD-50[b/]	5.25 mg/kg	Tucker and Haegele (1971)
ring-necked pheasant (*Phasianus colchicus*)	LC-50[a/]	1,075 ppm	Hill et al. (1975)
	Oral LD-50[b/]	53.4 mg/kg	Tucker and Haegele (1971)
mallard (*Anas platyrhynchos*)	LC-50[a/]	330 ppm	Hill et al. (1975)
	Oral LD-50[b/]	3.08 mg/kg	Tucker and Haegele (1971)
starling (*Sturnus vulgaris*)	Oral LD-50	7.5 mg/kg	Schafer (1972)
red-winged blackbird (*Agelaius phoeniceus*)	Oral LD-50	3.2 mg/kg	Schafer (1972)
chukar partridge (*Allectoris graeca*)	Oral LC-50[b/]	14.3 mg/kg	Tucker and Haegele (1971)
pigeon (*Columba livia*)	Oral LD-50[c/]	5.9 mg/kg	Tucker and Haegele (1971)
house sparrow (*Passer domesticus*)	Oral LD-50[b/]	12.6 mg/kg	Tucker and Haegele (1971)
chicken (*Gallus gallus domesticus*)	Oral LD-50[b/]	10.0 mg/kg	Abou-Donia (1977)

a/ LC-50 = median lethal concentration based on five days of exposure of approximately two-week old birds to treated feed, followed by three days exposure to untreated feed.
b/ Females only.
c/ Males and females.

Table 7. Summary of General Mammalian Toxicity Data of EPN

Test Species	Type of Study	Dose level Remarks	Results	References
rat	acute oral LD-50	-	7.8 mg/kg (female) 33 mg/kg (male)	Neal and DuBois (1965)
rat	acute oral LD-50	-	7.7 mg/kg (female) 36.0 mg/kg (male)	Gaines (1969)
rat	acute oral LD-50	-	37.5 mg/kg (female)	Suzuki (1973)
weanling rat	acute oral LD-50	-	8.0 mg/kg	Brodeur and DuBois (1963)
rat	acute dermal LD-50	-	25 mg/kg (female) 230 mg/kg (male)	Gaines (1969)
rabbit	acute dermal	200 mg/kg 2,000 mg/kg	no effect 4 of 4 died	Letter (1977b)
rat	acute (4-hr) inhalation	2.0 mg/liter 102 mg/liter	no effect 6 of 10 rats died within 4 hrs	Letter (1977b)
rabbit	eye irritancy	0.1 ml	no effect after 24 hrs; 3 of 8 died[a/]	Letter (1977b)
dog	chronic oral	0, 0.1, 0.5 and 2.0 mg/kg (1 year)	no effects other than tendency for increased liver weight with increasing doses	Hodge et al. (1954)
rat	chronic oral	0, 50, 150 and 450 ppm (male) and 0, 25, 75, and 250 ppm (female) for 2 years	no effect other than retarded growth at 450 ppm (male) and 225 ppm (female)	Hodge et al. (1954)
rat	chronic oral	0, 3, 15, and 75 ppm (male and female) for 6 months	depression of secretion of submandibular gland at 75 ppm at 3 months	Suzuki (1973)

a/ EPN is highly toxic via the ocular route without signs of eye irritancy.

4.5 kg/ha or more would be hazardous to sheep and cattle. The lowest recommended application rate for EPN (0.84 kg/ha) would probably be hazardous to calves.

(4) Toxicity of EPN Metabolites and Isomers

Dupont (1977b)[7/], in acute oral toxicity tests on rats using metabolites of EPN, reported approximate lethal dose (ALD)[8/] values of >5,000 mg/kg for ethyl phenylphosphonic acid, 2,250 mg/kg for ethyl phenylphosphonothioc acid, and 3,400 mg/kg for phenyl phosphonic acid.

Ohkawa et al. (1977) determined LD_{50} values for the optical isomers of EPN in mice, hens, houseflies, and rice stem borer larvae (Table 8).

Table 8. Toxicity of EPN Isomers to Mice, Hens, Houseflies, and Rice-Stem Borer Larvae

Species	Type of Administration	LD-50 Compound		
		(+) EPN	(-) EPN	(±) EPN [racemic]
Mouse (mg/kg)	intraperitoneal	17	16	16
Hen (mg/kg)	intraperitoneal	12	47	17
Housefly (ug/kg)	topical	1.1	3.2	1.3
Rice-stem borer (ug/kg)	topical	2.9	11.7	5.9

The authors reported that the (+) isomer of EPN exhibited a greater insecticidal toxicity to houseflies

7/ Studies submitted by registrants in support of tolerance petitions are automatically classified CONFIDENTIAL prior to action on the petition.
8/ The ALD was based on single doses of one animal per dose level by intragastric intubation.

(2.9X) and rice stem borer (4.0X) than the (-) EPN isomer. The (+) isomer was also found to be 3.9X as toxic to hens. Both isomers were reported to be equally toxic to mice. Similar results were obtained by Nomeir and Dauterman (Undated) in houseflies and mice using the optical isomers of EPN and EPNO.

I. Pesticide Episode Review System (PERS) Reports

A Pesticide Episode Reports System (PERS)[9/] is maintained by EPA's Pesticide Response Branch of the Office of Pesticide Programs (Memo 1976). PERS collects reports of pesticide exposure affecting humans, domestic animals, livestock, and wildlife.

From 1971 to 1976 six episodes were reported involving the pesticide EPN. Five of these episodes involved human exposure to EPN and other pesticides; one involved contamination of unspecified materials during transportation by EPN alone. Of the five cases involving humans, two reported normal cholinesterase levels, two did not report on cholinesterase levels, and one reported a cholinesterase level of 1.45 International Units (normal range, 2.45 to 5.04). All five of these cases involved exposure to other pesticides as well as to EPN.

9/ This system is now (1979) called the Pesticide Incident Monitoring System (PIMS).

II. REGULATORY HISTORY

EPN has been subjected to three regulatory actions, initiated during the period when USDA was responsible for the regulatory control of pesticides.

PR Notice 68-6 published on February 1, 1968, proposed the cancellation of use of EPN on onions in the absence of a finite tolerance or exemption (USDA 1968a).

PR Notice 68-8 published on April 24, 1968, classified certain chemical use patterns as non-food uses, allowing registration of products for these uses to continue in the absence of finite tolerances. Use of EPN as a special mosquito larvicide was classifed as a non-food use (USDA 1968b).

PR Notice 68-19 published on November 29, 1968, classified EPN and certain other pesticides as highly toxic to bees and required the label statement, "This product is highly toxic to bees exposed to direct treatment or residues on crops. Protective information may be obtained from your Cooperative Agricultural Extension Service" (USDA 1968c).

III. SUMMARY OF SCIENTIFIC EVIDENCE TO SUPPORT REBUTTABLE PRESUMPTION

A. Delayed Neurotoxicity

40 CFR 162.11(a)(3)(ii)(B) provides that a rebuttable presumption shall arise if a pesticide "[p]roduces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator,

which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety...." The Agency has concluded that all pesticides containing EPN exceed the chronic risk criterion relating to delayed neurotoxicity.

Delayed neurotoxicity is a phenomenon caused by some organophosphorous compounds such as tri orthocresyl phosphate (Smith et al. 1932) and leptophos (Abou-Donia and Preissig 1976). A characteristic clinical sign of this adverse effect in sensitive species is ataxia which usually appears several days after dosing or poisoning. Histopathologically, delayed neurotoxicity appears to be characterized by a dying back of the axonal region of the neuron with secondary damage to the myelin sheath (Johnson 1974). The target site in the central nervous system is believed to be a specific protein called neurotoxic esterase (Johnson 1975; El-Sebae et al. 1977). Because of its sensitivity, the chicken hen is the species of choice in neurotoxicity testing.

In assessing the risk of EPN to human health, the Agency has considered the lowest tested dosage (0.01 mg/kg per day) which has <u>not</u> produced either histological changes or clinical signs indicative of delayed neurotoxicity in the most sensitive species (no-observed effect level, or NOEL). Based on the exposure estimates discussed in Section III.A.(3) of this document, the Agency concludes that the anticipated

amount of EPN to which pesticide applicators and unprotected bystanders may be exposed by the dermal (and, in some instances, by the inhalation) route may not provide an ample margin of safety. The Agency also concludes that insufficient information exists on the exposure of the general population to EPN resulting from the consumption of residues on food to initiate a rebuttable presumption. The Agency requests registrants and other interested parties having information on EPN residues on food to submit such information to the Agency for consideration in the RPAR process. Finally, the Agency also requests registrants and other interested persons to comment on information the Agency has received which indicates that some applicators may ignore label directions to wear protective clothing.

(1) Hens

Abou-Donia and Graham (1978) administered technical grade EPN (85%) to 19-month-old, mixed-breed laying hens (Gallus gallus domesticus), weighing 1.57 ± 0.03 kg. The birds were placed in individual cages for one week to adjust to the environment before beginning the study. Seven groups of birds (six birds in each group) received single daily oral doses of 0.01, 0.1, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/kg of technical EPN in gelatin capsules for three months. Surviving birds were observed for an additional month after termination of the treatment. Four groups of controls were used. These were administered empty gelatin capsules,

atropine sulfate (only for 34 days), parathion (negative controls), and tri-o-cresyl phosphate (TOCP - positive controls), respectively.

All hens given doses of 2.5, 5.0, and 10.0 mg EPN/kg showed acute cholinergic signs shortly after administration of the first dose. All subsequent administrations of EPN at these dosages were preceded by oral administration of atropine sulfate in order to avert the pesticide's acute effects.

Five to 21 days after the initial dose, ataxia was observed in all hens receiving EPN at 0.1 to 10 mg/kg. Ataxia became evident an average of 19 days after administration at the 0.1 mg/kg level, compared to 8.6 days at the 10.0 mg/kg level. Hens given 0.01 mg/kg showed no abnormalities throughout the experiment.

The severity of the clinical effects was dose dependent. The clinical condition of four of the six hens that were dosed with 10 mg EPN/kg per day progressed from paralysis at 7 to 66 days, to death at 8 to 67 days. One hen died shortly after the first dose without paralysis, and the sixth hen demonstrated severe ataxia. Four of the six hens administered 2.5 mg EPN/kg per day experienced ataxia with near paralysis. Two hens demonstrated severe ataxia characterized by legs sprawling out in front, inability to stretch the legs, and lowering of the hind quarters. At the 0.5 mg/kg level, five hens exhibited total ataxia (characterized

by a disturbance of control of leg movement with a change in gait), and one hen progressed to severe ataxia. At the 0.1 mg/kg, the clinical condition of five hens progressed to total ataxia. Three days after the last dose of EPN, the condition of all five hens improved to mild ataxia (characterized by diminished leg movement, reluctance to walk, and the tendency to slide on the floor and fly). However, the mild ataxia persisted during the remaining 27 days of the observation period. The condition of the sixth hen progressed to paralysis and death.

No paralysis or ataxia was observed in any of the control hens administered empty gelatin capsules, atropine sulfate alone, or EPN at 0.01 mg/kg. All negative control hens that were given parathion showed leg weakness. However, except for one hen which died, all completely recovered after administration of parathion was stopped. All the positive controls developed paralysis or near paralysis.

The most consistent histologic abnormalities were observed in the anterior column of the spinal cord. All examined hens which received 5 and 10 mg EPN/kg per day and two hens which received 2.5 mg EPN/kg per day exhibited severe lesions characterized by loss of myelin; absence of both axons and myelin in scattered areas; swollen, frayed, and fragmented axons; and occasional reactive astrocytes. Posterior columns and sometimes lateral columns of the spinal cord exhibited occasional swollen,

fragmented axons without detectable myelin loss. Another two hens from the 2.5 mg/kg group and two hens from the 1.0 mg/kg group were found to have occasional swollen and fragmented sheaths and axons in the spinal cord. No histologic abnormalities were found in the remaining four hens in the 1.0 mg/kg group, any of the six hens in the 0.5 mg/kg group, nor in five hens in the 0.1 mg/kg group. One hen in the last group showed perivacular lymphocytic cuffing in the thoracic cord, but no sign of neuronal or myelin degeneration.

Mild axon and myelin degeneration in the sciatic nerve was observed in one hen administered 10 mg EPN/kg per day. One hen given 5.0 mg EPN/kg per day exhibited rare swollen or missing sheaths and axons in the sciatic nerve. No lesions were observed in the examined brains or retina and optic nerves of the EPN-treated hens.

All positive control hens were observed to have spinal cord lesions similar to those observed in EPN-treated hens. No histological abnormalities were observed in the spinal cord, brain, or sciatic nerve of hens treated with empty gelation capsules, parathion, or atropine sulfate.

In an unpublished experiment described in a preliminary report by Sakamoto (1977), oral doses of 1, 3, and 10 mg/kg of EPN in aqueous suspension were administered to three groups of White Leghorn laying hens (ten hens in each group) every day except Sunday for 28 days. The hens were observed for 21 days after administration.

Four of the ten hens fed the highest dosage of EPN demonstrated mild ataxia beginning from 23 to 42 days after the first day of administration. It appears from the preliminary report that none of the affected hens recovered during the observation period. No ataxia was observed in any of the hens fed 1 or 3 mg EPN/kg or in any of the negative controls. All ten positive controls, fed 50 mg/kg per day of TOCP, demonstrated paralysis. Although histological specimens of nerve tissue of all hens were taken, the results were not available when the preliminary report was prepared.

A 120-day feeding study of the effects of EPN in the diets of adult female chickens of the Rhode Island Red strain is summarized in a Communicable Disease Center publication (CDC Undated). Groups of two chickens were fed ground Purina Laboratory Chow containing 0, 20, 50, 100, 200, and 400 ppm of technical EPN. The two hens on the 400-ppm dose refused to eat the EPN-contaminated diet, and both died on the 45th day, presumably of starvation. The authors noted muscle weakness in the legs in four chickens fed approximate average daily doses of 0.6, 1.1, 1.8, and 2.4 mg EPN/kg beginning on the 25th, 50th, 42nd, and 42nd day of the experiment, respectively. This trait persisted in all cases until death or until the end of the 120-day feeding study. The summary made no mention of whether leg weakness appeared in the two controls or the remaining four

birds fed an average of 0.6, 1.0, 2.3, or 3.4 mg EPN/kg per day over the course of the 120-day feeding period. A histopathological examination was not performed on any of the birds.

Durham et al. (1956) dosed ten atropinized adult hens of the Rhode Island Red Strain with a single 60 mg/kg subcutaneous dose of EPN in peanut oil carrier. Leg weakness occurred immediately in all hens. The minimum dosage at which this trait was noted was 40 mg/kg (number of hens at this level not indicated). The muscle weakness following dosage of EPN was irreversible in most cases, with one survivor still showing leg weakness 308 days after dosing.

Witter and Gaines (1963) dosed thirteen hens of a sex-linked [sic] strain with a single 60 mg/kg subcutaneous dose of EPN in peanut oil carrier. These workers observed paralysis lasting from the first day to the termination of the experiment (unspecified period).

Aldridge and Barnes (1966) administered a single dose of ethyl p-nitrophenyl phenylphosphonate (EPNO) to six-month-old hens of a Rhode Island Red X Light Sussex cross by subcutaneous injection using ethanol as the carrier. As discussed previously in Section I.E.(1), EPNO has been indicated as a possible metabolic product of EPN in mammalian systems. At a dosage of 10 mg/kg EPNO, some birds exhibited ataxia characteristic of delayed neurotoxicity. The article

also reported that histologic lesions as described in another article (Cavanagh 1954) were observed. The number of birds tested, the number exhibiting clinical symptoms, and the exact nature of the clinical and histological findings were not discussed in the Aldridge and Barnes study.

Gaines (1969) administered single subcutaneous doses of EPN and other carbamate and organophosphorous compounds to atropinized chickens. The various compounds were tested in either White Leghorn or Rhode Island Red chickens, but the strain used for the EPN study was not indicated. The lowest dose of EPN which produced leg weakness in the chicken was 40 mg/kg and the highest ineffective dose was 20 mg/kg. Some of the chickens exhibiting leg weakness failed to recover completely during the observation period (greater than 330 days). The number of chickens used in the study and the percentage of chickens which exhibited leg weakness were not indicated.

Ohkawa et al. (1977) administered single intraperitoneal doses of the optical isomers of EPN in dimethyl sulfoxide solution to White Leghorn chickens (1.7 to 2.2 kg). The hens were atropinized immediately, and 6 and 24 hours afterwards. The various doses used and the results observed are given in Table 9.

Table 9. Delayed Neurotoxicity of Racemic EPN, (+) EPN, and (-) EPN in Hens

Compound	dose (mg/kg)	hens dosed, No.	death No.	delayed paralysis No.
(+) EPN	31.2	3	0	0
	40.6	3	0	0
	52.8	3	0	0
	68.6	3	0	0
	89.2	3	2	0
(-) EPN	31.2	3[a/]	0	0
	40.6	3[a/]	1	2
	52.8	3[a/]	2	1
	68.6	3	0	3
	89.2	3	0	3
racemic (±) EPN	31.2	5	0	0
	40.6	5	0	0
	52.8	5	1	1
	68.6	3	0	1
	89.2	3	1	2

a/ Hens were not atropinized.

Paralysis of the legs developed in the hens dosed with (-) EPN (all surviving hens at all doses except the lowest) and racemic (±) EPN (four hens at the higher doses). The paralysis occurred 10 to 14 days after administration and appeared to be irreversible, persisting to the end of the four week observation period. No paralytic signs were seen in hens dosed with the (+) isomer at any level. The authors did not indicate whether these hens exhibited any ataxia.

The histological examination showed degenerating myelin sheaths and swollen and fragmented axons in the sciatic nerve, and cervical, thoracic, and lumbar regions of the spinal cord of (-) EPN poisoned hens. The (+) EPN

treated hens did not demonstrate these changes, and the authors did not indicate whether any histological examination of the (±) EPN treated hens was performed.

The Biochemicals Department of the E.I. du Pont de Nemours and Company (DuPont 1977a) has submitted to EPA a progress report on its ongoing two-year chronic oral toxicity study on chickens. Six groups of 50 White Leghorn hens were fed a daily diet containing 0 (negative control), 1, 5, 15, and 45 ppm of technical EPN and 200 ppm TOCP (positive control). After 31 weeks, 38 of the 45 surviving hens fed 45 ppm EPN exhibited clinical signs of neurotoxicity ranging from slight intermittent ataxia (20 hens) to moderate or severe ataxia (8 hens). One hen was unable to stand due to paralysis of the legs. Ataxia first appeared after seventeen weeks, although the preliminary report is not clear on this point. No clinical abnormalities were observed after 31 weeks in the controls or in the hens fed 1, 5, and 15 ppm EPN. Thirty-seven of the surviving forty-four positive controls exhibited clinical signs of gross neurotoxicity (DuPont 1977a). Preliminary histopathological examinations were performed at 50 weeks (DuPont 1979a) on three hens in each group, except for the 45 ppm group in which four hens were sacrificed. The Agency's analysis of this study awaits completion of final histology, residue analysis, and biochemical measurements, which are in progress. DuPont reported that treatment-related changes were observed in the

spinal cords and brains of hens in the 45 ppm group; no lesions atrributable to EPN were noted in hens from the 1, 5, or 15 ppm groups. No treatment-related changes were evident in the sciatic nerves of any dose group. Electron microscope histology of nerve tissues from hens at the 45 and 15 ppm levels revealed certain alterations in the sciatic nerve but not in the brain or spinal cord. DuPont also noted that these lesions might have resulted from poor fixation and improper handling of tissues in preparation for electron microscopy. It is not possible to determine a no-effect-level for EPN from the results of the DuPont study because the study has not yet been completed and because the progress reports lack sufficient detail.

In a paper presented at a conference on delayed neurotoxicity, Frawley (1976) reconstructed from his laboratory records the results of unpublished neurotoxicity studies he and his coworkers had conducted twenty years before. In one study, he had administered a diet containing approximately 300 to 600 ppm of EPN daily to four hens (probably of the Rhode Island Red strain) over a period of five to fourteen weeks. The hens exhibited progressive muscular weakness and ataxia with eventual inability to stand. Histological examination of the EPN-treated hens revealed fragmentation and lysis of axons, swelling of nerve fibers, and myelin degeneration in the sciatic nerve. Controls showed no clinical or histological abnormalities.

In another study, hens were fed various doses of EPN over a seven month period. Three or four birds fed 50 ppm EPN, the lowest dose administered, demonstrated slight to moderate clinical signs indicative of delayed neurotoxicity. The histological reports for this study were lost.

Industrial Biotest Laboratories (1976) administered by gavage a single 28.8 mg/kg dose of EPN to twenty hens once at the start of the experiment and again 21 days later. The hens were observed for an additional 21 days. These hens exhibited severe ataxia, severe lethargy, and anorexia within 30 minutes of each dose of EPN. However, the report of the experiment states that the majority of the survivors were normal within 48 hours of each dose and that no behavioral signs indicative of neurotoxicity were noted. Histopathological examination of the brain, spinal cord, and sciatic nerve did not reveal any axonal degeneration or demyelination in any of the test hens. Positive control hens administered a single 500 mg/kg dose of TOCP exhibited extreme weakness of the legs and wings by the eleventh day after dosing. In addition, all the positive controls exhibited some axonal degeneration or demyelination in the spinal cord or sciatic nerve.

Although no signs of delayed neurotoxicity were observed in hens exposed to EPN in this study, this result is not necessarily inconsistent with the results obtained

in the experiments described above. All of those experiments elicited delayed neurotoxicity either after multiple exposures to lower levels or after single exposure to higher levels of EPN than were employed in the Industrial Biotest Laboratories study (1976).

Kaneko and Sakamoto (1976) administered oral doses of EPN ranging from 22.2 mg/kg to 48.7 mg/kg once at the start of the experiment and again 21 days later to six groups of five White Leghorn hens. No paralysis was observed in any of the surviving hens. However, the authors do not indicate whether any ataxia was observed. In addition, no description was given of the clinical status of any of the hens which died during the course of the experiment.

In assessing the margin of safety for humans, the Agency used the no-observed effect level [0.01 mg/kg] in the most sensitive species [chickens] (Abou-Donia and Graham 1978). Although Sakamoto (1977) showed no delayed neurotoxic effects in hens at dosages below 10 mg/kg, it is possible that his experiment was too short for these effects to be manifested at lower dosages. Furthermore, since EPA's Criteria and Evaluation Division [10/] has reviewed the Abou-Donia study and concluded that it was a well-conducted

10/ During an Agency reorganization, the Criteria and Evaluation Division (CED) was changed to the Human Effects Division (HED).

experiment, there is no reason to reject its findings. The other studies on delayed neurotoxicity were either single-dose experiments which provided no data on possible adverse effects at lower doses after multiple exposures, or did not provide sufficient information to determine the exact amount of EPN administered.

(2) Rodents

El-Sebae et al. (1977) administered a single oral dose of 20 mg/kg EPN in corn oil carrier to an unspecified number of adult white mice weighing 10 to 18 grams. Irreversible ataxia of the hind legs was seen after 29 days. The authors did not state how long the animals were observed.

In a preliminary study, Metcalf (Letter 1977c) fed a single dose of EPN in corn oil to three female rats at one-fourth the LD_{50} value (i.e., 10 mg/kg). Marked ataxia in the hind legs was noted on day 2 of the experiment. By day 6, the rats were normal, and no recurrence was noted one month later.

(3) Exposure Analysis

In order to determine whether a rebuttable presumption should be issued on the basis of delayed neurotoxicity under Section 162.11(a)(3)(ii)(B), the Agency must determine whether an ample margin of safety exists between the no-observed effect level for EPN-induced delayed neurotoxicity in test animals (0.01 mg/kg per day) and the level(s) to which humans can reasonably be anticipated to be exposed

through oral, dermal, or inhalation exposure. The following estimates of dermal and inhalation exposure are based on an analysis performed by EPA's Hazard Evaluation Division [HED] (Memo 1979c) of a study submitted by a registrant (Velsicol 1979). The levels of exposure were calculated for a 70-kg person.

(a) Oral Exposure

EPN is registered for use on a large number of raw agricultural commodities. Very limited monitoring data on EPN residues in food products are available [see Section I.G.(3)]. In addition, it appears that current use of EPN on food crops is either non-existent or negligible (Memo 1979a). Use of EPN on cotton, however, seems to be increasing (Memo 1979a; see also Section I.B). Cottonseed meal and oil contribute to the human diet (e.g., as salad oil or margarine). A tolerance of 0.5 ppm has been established for EPN residues in or on cottonseed. In order to determine possible dietary exposure from EPN residues on cottonseed, the Agency used the assumption that residues would be present at tolerance levels. Human dietary exposure to EPN would therefore equal 0.5 ppm [= 0.5 mg/kg] X 1.5 kg/day [total daily food intake] X 0.0015 [% contribution of cottonseed to daily diet] / 70 kg [adult weight] = 0.00002 mg/kg body weight per day. The Agency concludes that an ample margin of safety exists between exposure to EPN residues on cottonseed and the NOEL of 0.01 mg/kg.

Although it appears that there is currently little use of EPN on food crops (and therefore little exposure from EPN residues on food), the Agency points out that the potential for such exposure still exists because EPN use on food crops could increase in the future. The Agency has calculated possible future dietary exposure to EPN, based on the worst-case assumptions that residues would occur on all food crops for which EPN is registered and that residues would occur at tolerance levels (Memo 1979b). Possible future dietary exposure could therefore equal 0.016 mg/kg body weight per day. If EPN use on food crops becomes substantial in the future, then it is possible that residue levels on these crops would not constitute an ample margin of safety. The Agency is therefore considering possible regulatory action to ensure that an ample margin of safety will exist. The Agency therefore requests registrants and other interested parties to submit any information they may have on EPN residues on food crops, or on degradation of EPN residues.

(b) Inhalation and Dermal Exposure*/

The population most at risk for inhalation and dermal exposure are personnel involved in aerial and ground application of EPN (including airplane pilots, flaggers, and mixers and loaders of the formulated product), field workers and scouts who enter treated fields soon after application, and unprotected bystanders in contiguous fields or in adjacent dwelling places and other structures. The Agency has concluded that EPN exposure to these workers and to unprotected bystanders does not provide an ample margin of safety. The Agency is therefore issuing a rebuttable presumption against registration for all EPN products based on the "other chronic effects" (delayed neurotoxicity) risk criterion. In addition, the Agency is requesting registrants and other interested persons to submit comments or information on the failure of applicators to wear protective clothing,

*/ The Agency has also received an epidemiological study (DuPont 1979b) of workers in two DuPont plants where EPN is manufactured. This study, particularly the underlying medical data of the workers, is classified CONFIDENTIAL. The results of routine, as well as detailed, neurologic examinations performed on selected EPN workers who had the longest period of exposure (and thus the greatest likelihood of illness) did not reveal any findings of neurologic disease or deficit. DuPont pointed out that the levels of EPN in the monitoring studies at both plants were below OSHA's threshold limit value (TLV) for EPN of 0.5 mg/m^3. The Agency has not yet completed its review of this study; the Agency's analysis of this study will be included in Position Document 2/3. The Agency points out, however, that (assuming DuPont's data are correct) the results apply only to exposure in the manufacturing situation and not to exposure to the general population, applicators, or the environment from commercial or private use of EPN.

according to label directions, and on possible exposure to EPN of scouts and other agricultural field workers who re-enter treated fields 24 to 48 hours after application.

The Agency has recently received an EPN exposure study of applicators and support personnel (Velsicol 1979). This study presents data for inhalation and dermal exposure to pilots, mixers and loaders, flaggers, and ground spray applicators. Denim patches were attached to various parts of the body to measure dermal exposure, except that hand rinses with 1:1 mixture of acetone and water were used to measure dermal exposure of the hands. Personal air monitoring samplers were used to collect air from the breathing zones of workers to measure inhalation exposure.

The highest route of exposure was found to be the dermal route, which is consistent with a great deal of other exposure data available to the Agency. Recoveries of EPN from spiked denim patches in the laboratory were about 90%, but no data were presented on the retentive and/or absorptive capacity of EPN by the patches under field conditions. The usual material for patches for purposes of measuring dermal exposure is multiple layers of gauze or alpha-cellulose (Durham and Wolfe 1962). Therefore, it is conceivable that the reported dermal exposure may have been underestimated by the use of a single layer of denim. In addition, the published method for hand rinsing (Durham and Wolfe 1962) used 95% ethyl alcohol rather than 50% acetone,

so the reliability of these data is not clear. No data on urinary excretion of EPN or its p-nitrophenol metabolite were presented, so the Agency has no means available to estimate the actual rate of skin penetration of EPN.

In spite of these reservations about this study, the Agency believes that it represents the best information currently available on applicator exposure to EPN. In addition, the reported data are in some cases similar in magnitude to other data in the scientific literature on exposure [e.g., 1.18 mg/hr dermal exposure to pilots (Jegier 1964) compared with 0.64 mg/hr in Velsicol study]. Accordingly, exposure data from the Velsicol study for those portions of the body which might normally be uncovered (back and front of neck, hands, and forearms) will be used to estimate dermal exposure. (Although label directions require that applicators wear protective clothing and respirators, information has recently come to the Agency's attention which indicates that at least some workers in some parts of the country may ignore these label directions to wear protective clothing because of hot weather [Letter 1978c].) Inhalation exposure values were estimated by using a breathing rate of 1.8 m^3/hr. The dermal and inhalation exposures for a 70-kg worker, calculated by the Agency from Velsicol's exposure data, are presented in Table 10 (Memo 1979c).

Table 10. Dermal and Inhalation Exposure to EPN of Applicators and Support Personnel During Application to Cotton

Workers	Dermal Exposure (mg/kg per hr)		Inhalation Exposure (mg/kg per hr)	
	High	Low	High	Low
Pilots (4)*	0.009	0.0016	0.000023	0.000015
Loaders (3)*	0.026	0.0015	0.000032	0.000019
Flaggers (3)*	0.46	0.053	0.021	0.000025
Ground Applicators (2)*	0.028	0.0038	0.00012	0.000021

* Number of workers monitored. High and low exposure values are based on the highest and lowest exposures for the workers monitored.

(c) Conclusions

The Agency makes the following observations about these exposure values:

1) Based on an NOEL of 0.01 mg/kg per day, none of the high or low dermal exposure values present an ample margin of safety, for applicators chronically exposed;

2) Based on an NOEL of 0.01 mg/kg per day, two of the high inhalation exposure values (for flaggers and ground applicators) do not present an ample margin of safety for applicators chronically exposed;

3) Cumulative exposure to EPN through two or more routes of exposure (oral, dermal, inhalation) will not present an ample margin of safety in most situations (i.e., dermal + oral, dermal + inhalation, or high inhalation + oral); and

4) The most restrictive instructions appearing on the labeling of registered EPN products provide only that unprotected persons be kept away from treated areas where

there is danger of drift. The Agency considers these instructions too vague to assure that the application of EPN does not occur close to unprotected persons, including those in contiguous fields and those in adjacent dwelling places and other structures. It is anticipated that some of these persons may be exposed to levels of EPN as high or higher than that to which applicators and support personnel may be exposed on the day of application. In addition, because EPN is applied several times during a growing season to certain crops, it follows that some people located near application sites may be exposed several times during the same time period.

The Agency is therefore issuing a rebuttable presumption against registration of all EPN products on the basis that applicators, support personnel, and unprotected bystanders may be exposed to levels of EPN which do not assure an ample margin of safety.

The Agency notes that Velsicol (1979) suggested several methods of reducing dermal and inhalation exposure to EPN. The Agency will consider these suggestions as possible regulatory options, if the presumption of risk against EPN is not rebutted. In addition, information has come to the Agency's attention (Letter 1978c) that some pesticide applicators in some areas, particularly during hot weather, may ignore label directions to wear protective clothing and/or respirators. The Agency requests registrants

and other interested persons to submit any information they may have on whether such practice is common or widespread. The Agency also recognizes that there are other agricultural field workers (e.g., cotton scouts) who are likely to be exposed to EPN after application. The Agency requests registrants and other interested persons to submit any information they may have on this issue to the Agency.

(d) Cumulative Exposure

The Agency also notes that possible multiple exposure to several pesticides which cause delayed neurotoxicity could increase the total body burden and increase total risk from such exposure. Since there is insufficient information available on possible multiple exposure, the Agency is not now issuing a rebuttable presumption on this basis. The Agency does request registrants and other interested persons having information on possible multiple exposure to several neurotoxic pesticides to submit such data to the Agency. Specifically, the Agency requests information on whether those pesticides with which EPN is formulated may cause neurotoxic effects.

B. Acute Hazard to Wildlife: Aquatic Species

40 CFR Section 162.11(a)(3)(i)(B)(3) provides that a "rebuttable presumption shall arise if a pesticide's [use]...[r]esults in a maximum calculated concentration following direct application to a 6-inch layer of water more than 1/2 the acute LC_{50} for aquatic organisms representative

of the organisms likely to be exposed as measured on test animals...."

EPN is registered for use by Mosquito Abatement Districts, Public Health Officials, and other trained personnel of Public Mosquito Control Programs as a mosquito larvicide (Memo 1978a). Recommended application rates for EPN as a mosquito larvicide are 0.05 to 0.1 pound active ingredient/acre using ground or aerial equipment. The higher rate is recommended in areas where mosquitoes have shown resistence to other organophosphates. Although two of the three EPN products registered for use as a larvicide may not, according to label directions, be used in lakes, ponds, streams, or other bodies of water, the label directions are not sufficiently precise to assure that aquatic species will not be exposed. The label for the other larvicide product does not prohibit the application of this product to bodies of water.

Application of EPN at the lowest recommended rate for larvicidal uses (0.05 pounds AI/acre) will result in a concentration of 36.7 ppb in a 6-inch layer of water (Memo 1978a). This dosage will exceed 1/2 the LC_{50} or TLm for all but one (bluegill sunfish) of the aquatic species listed in Table 11. Application at the highest recommended rate (0.1 pounds AI/acre) will result in a concentration of 73.4 ppb in a 6-inch layer of water (Memo 1978a), which exceeds 1/2 the LC_{50} of the bluegill sunfish

in addition to the other listed species. Accordingly, the Agency is issuing a rebuttable presumption against all pesticide products containing EPN which are registered for direct application to water.

Table 11. Half LC-50 or TLm Values for Representative Aquatic Species Likely to be Exposed to EPN Mosquito Larvicide

Species	Exposure Time/-Toxicity Parameter	Toxicity Value (ppb)	1/2 Toxicity Value (ppb)	Reference
Juvenile striped bass (Morone saxatilis)	96 hr/TLm[a/]	60	30	Korn and Earnest (1974)
spot (Leistomus xanthurus)	96 hr/LC-50[b/]	25.6	12.8	Schimmel et al. (1978)
bluegill sunfish (Lepomis macrochirus)	96 hr/LC-50	80	40[c/]	Letter (1977b)
Pinfish (Lagodon rhomboides)	96 hr/LC-50	16.5	8.2	Letter (1978)
scud (Gammarus lacustris)	96 hr/LC-50	15	7.5	Sanders (1969)
pink shrimp (Penaeus duorarum)	96 hr/LC-50	0.29	0.15	Schimmel et al. (1978)
daphnia (Daphnia magna)	48 hr/LC-50	0.32	0.16	Letter (1977b)
daphnia (Daphnia pulex)	3 hr/TLm	1.2	0.6	Nishiuchi and Hashimoto (1967)
daphnia (Moina macrocopa)	3 hr/TLm	7.1	3.55	Nishiuchi and Hashimoto (1967)

a/ Median tolerance limit.
b/ Lethal concentration to 50% of the population
c/ Exceeds half toxicity value at 0.1 but not at 0.05 pounds/acre application rate.

IV. OTHER RELEVANT ADVERSE EFFECTS

EPN has been demonstrated to cause other adverse effects. However, these effects either occur at levels well above which humans may reasonably be anticipated to be

exposed, taking into account an ample margin of safety, or are not sufficiently documented by the available data to warrant issuance of a presumption at this time. The Agency solicits any additional information which indicates that the use of EPN pesticides may result in the effects discussed below or any other adverse effects.

A. Teratogenic and Muscular Effects

On the thirteenth day of incubation, just prior to the appearance of cholinesterase, Khera et al. (1965) inoculated the yolk sacs of duck eggs with doses of 10 and 100 ug/egg of technical EPN dissolved in 0.1 ml of propylene glycol. Thirty-four percent of the ducklings hatched from eggs treated with 100 ug EPN were severely affected and displayed signs of asthenia and lethargy. Of these, 27% exhibited permanent malformations of the leg and foot involving axial rotation of one or both feet. Extreme cases exhibited talipes varus and talipes calcaneovarus, most often involving the metatarsophalangeal and phalangeal levels. Three of the ducklings (11%) hatched from eggs treated with 10 ug EPN exhibited malformations of the foot, but these malformations were transient.

A year later Khera et al. (1966) reported on the pathology of pre- and posthatched skeletal leg muscle and enzymatic alterations in embryonic thigh muscle in embryos of hatched ducklings inoculated via the yolk sac with 100 ug/egg of EPN dissolved in 0.1 ml of propylene glycol.

X-ray studies indicated that the primary cause of talipes varus was not change in the leg bones when compared to controls. Rather, this malformation was found to be associated with dystrophic changes in the thigh and shank muscles. Cholinesterase was reported to be progressively inhibited at the motor end plates in the thigh skeletal muscle during embryogenesis.

Yamada (1973) demonstrated dyskinesia of the legs of male chicks incubated from eggs whose whites had been inoculated with 1 mg/egg to 20 mg/egg of 45% EPN emulsifiable concentrate [solvent(s) and inert component(s) undisclosed].

In addition to these studies, negative teratology studies have been reported by Upshall et al. (1968), Proctor et al. (1976), and Flockhart and Casida (1972).

B. Cholinergic Effects

EPN, like most organophosphates, inhibits the action of acetylcholinesterase (AChE). This inhibition results from an irreversible chemical reaction between EPN and the enzyme. The return of AChE activity occurs only as new enzyme is synthesized and may take days or months. The AChE inhibition prevents the breakdown of acetylcholine and results in a variety of cholinergic effects. Although the symptoms vary according to the dosage, some of the signs which may be expected include constriction of the iris sphincter and ciliary muscle in the eye; increased motor activity of the gastrointes-

tinal tract; fibrillations, fasciculations, weakness, and eventual paralysis, at very high dosages, of the voluntary muscles; increased secretion of the various glands; and contraction of the bronchioles (Goodman and Gilman 1970).

In the previously cited study by Abou-Donia (1977), cholinergic signs were observed in hens fed EPN at dosages as low as 2.5 mg/kg per day immediately after administration. As expected, these symptoms disappeared upon administration of atropine. The author did not describe the exact nature of the signs observed.

Moeller and Rider (1962) administered daily oral doses of EPN to adult human male volunteers. Doses of 3, 6, and 9 mg EPN per day were administered to three groups of five subjects for up to 47 days. No clinical effects were observed at any dose level. Those men receiving 9 mg per day (approximately 0.1 mg/kg per day), however, demonstrated a significant depression of plasma pseudocholinesterase and red blood cell cholinesterase activity. No such depression occurred at the two lower doses.

C. Disorders of the Eye

A few Japanese studies suggest that exposure to EPN can result in various eye disorders. In one study, Ishikawa et al. (1971) examined 71 children from an area of Japan where EPN and several other organophosphate pesticides were used. These researchers observed the

following percentages of eye abnormalities in the subjects: reduced vision, 98%; narrowing of peripheral visual field, 95%; abnormal refraction, 88%; oculogyration disorder, 57%; pupillary disturbances, 52%; optic and retinochoroidal atrophy, 65%; accommodation paralysis, 12%; and nystagmus, 6%. The pathogenesis of these disturbances is not clearly described by the authors. It appears possible, however, that a combination of cholinergic effects, delayed neurotoxicity, and direct effects on the eye may be involved. It is impossible to draw any conclusions from this study about the possible hazards which might result from EPN use in the United States. First, the study does not indicate the extent of the children's exposure to EPN. In addition, the children were apparently exposed to several pesticides, and the routes of exposure are described only vaguely.

Other case histories (Ozawa et al. 1972) have been reported from Japan, indicating that persons exposed to EPN and other organophosphate pesticides have developed opthalmic disorders similar to some of those described by Ishikawa et al. (1971).

D. Possible Mutagenic Effects

Amer and Ali (1969) demonstrated that lateral root cells of Vicia faba seedlings treated with p-nitrophenol (a metabolic breakdown product of EPN) produced a lengthened metaphase stage. This effect resulted in the accumulation

of cells in the mitotic stage in treated seedlings greater than that seen in untreated plants.

Fahrig (1974) reported that p-nitrophenol showed weak genetic activity in a liquid holding test for mitotic gene conversion in S. cerevisiae. Four other tests on the mutagenic activity of p-nitrophenol were negative.

E. Reductions in Populations of Nontarget Organisms

Eckert (1950) reported that 50% of an undisclosed number of bees fed 0.02 cc of syrup containing 0.12 mg technical EPN died within 24 hours and that 50 bees exposed to 0.02 gram of a 27% EPN dust for five minutes were moribund within an hour.

In laboratory toxicity tests Anderson and Tuft (1952) found that confinement of bees in cages with bouquets of flowers (Lippia lanceolata) dusted with 2% EPN resulted in 100% mortality within five hours. In laboratory tests where direct application of 2% EPN to honeybees was made, the percentage mortality at 10, 20, 30, and 40 minutes was 5, 30, 90, and 100%, respectively. The authors rated EPN as highly toxic.

Anderson and Atkins (1967) measured the relative toxicity of 237 pesticides to honeybees in California from 1950 to 1966. EPN was categorized as highly toxic with an LD_{50} contact toxicity value of less than 2 ug/bee as determined by laboratory and field tests predominantly on alfalfa, cotton, peaches, citrus, ladino clover, and sweet corn.

Atkins et al. (1973) determined that the 48 hour LD50 value of EPN to honey bees in laboratory tests was 0.245 ug/bee at 26.7°C (80°F) and 65 percent relative humidity.

Although the studies cited here indicate the extreme toxicity of EPN to honeybees, currently registered EPN products are required to print a warning of this hazard on the label (See Section II). Since the Agency does not know if current use patterns of EPN are likely to result in exposure of a significant number of bees to lethal concentrations of the pesticide, the Agency is not now presuming against EPN on this basis.

V. Potentiation of Other Compounds by EPN

Potentiation occurs when the observed effects of administering two or more compounds are greater than the sum of the effects when the individual chemicals are given alone (Fitzhugh 1966). Potentiation of the acute toxicity and cholinesterase depressive effect of malathion, dimethoate, and systox by EPN has been observed in the studies discussed below. Although all four chemicals do have certain use patterns in common, the Agency does not know if it is common practice in the United States to mix dimethoate, systox, or malathion with EPN, or if these pesticides are applied simultaneously or in quick succession. The Agency requests registrants and other person with information on this matter to submit such information to EPA. If it appears that EPN is used in such a manner which results

in simultaneous exposure to EPN and any of the other three pesticides, EPA will further evaluate the risks posed by potentiation to determine if labeling changes prohibiting or restricting such usage are warranted.

EPN is formulated in separate combination with parathion, methyl parathion, toxaphene and Guthion. However, the Agency has no data indicating whether EPN potentiates or is potentiated by any of these pesticides. The Agency requests registrants and any other persons possessing information on this matter to submit such data to the Agency.

A. Malathion

Potentiation between EPN and malathion in biological systems is well documented. Frawley et al. (1957) demonstrated that 1/40 of the LD_{50} of malathion was lethal to rats and dogs by the oral route in the presence of subtoxic oral doses of EPN. Klaassen et al. (1959) found an oral LD_{50} in male Swiss white mice of about 13% of the LD_{50} of each compound separately. Similar results have been reported by several other workers (see Seume and O'Brien 1960a; Karczmar et al. 1962; Casida et al. 1963; Kreitzer and Spann 1973; and Macek 1975).

Detoxification of malathion involves the hydrolysis of carboxyester or carboxyamide linkages by tissue or plasma aliesterases (also termed carboxylesterase). Some researchers believe that EPN potentiates malathion by

inhibiting the aliesterases which normally detoxify malathion (Cook et al. 1958; Murphy and DuBois 1957; and Seume and O'Brien 1960b). However, not all observers agree on this mechanism and some inconsistencies with it have been noted (Bhagwat and Ramachandran 1975; Cohen and Murphy 1971; and Karczmar et al. 1962).

Although there are no registered products which contain both EPN and malathion, these chemicals do have many uses in common. These include use on apples, apricots, beans, beets, blackberries, boysenberries, cherries, citrus fruits, corn, dewberries, grapes, lettuce, loganberries, nectarines, peaches, pears, pineapples, plums, quinces, raspberries, rutabagas, soybeans, spinach, strawberries, sugar beets, tomatoes, turnips, and various nuts. There is no definite evidence that these pesticides are used together in the United States. However, Komoto et al. (1973) reported that, according to agricultural practice in certain rice fields in Japan, malathion sprays are used from March 15 to May 15 and EPN from April 15 to June 15. Thus, from April 15 to May 15 EPN and malathion are used together in the rice fields.

B. Dimethoate

Uchida et al. (1966) found that 1/2 the LD_{10} of EPN (11 mg/kg) and 1/2 the LD_{10} of dimethoate (170 mg/kg) killed an average of 90% of mice in two trials when given simultaneously. For guinea pigs, the authors found that 1/2 the LD_{10} of

dimethoate (390 mg/kg) and 1/2 the LD_{10} of EPN (14 mg/kg) killed an average of 24% ± 5% in five trials when given simultaneously. The authors concluded that the inhibition of the hepatic metabolism of dimethoate by EPN accounted for this synergistic activity.

Although there are no registered products which contain both EPN and dimethoate, these chemicals do have certain use patterns in common. These include use on apples, beans, cotton, grapes, pears, pecans, and tomatoes.

C. Systox

Williams et al. (1958), in a six-week dietary study, reported on the in vivo effects of a paired combination of EPN and Systox on serum and red blood cell cholinesterase levels in dogs. When levels of 2.0 ppm Systox and 20.0 ppm EPN were fed, an average depression in plasma cholinesterase to 44% of the pretreatment control occurred. This value is below the expected depression level, based on earlier studies, if the effect were additive. This indicates that EPN may potentiate the action of Systox in depressing plasma cholinesterase.

Although there are no registered products which contain both EPN and Systox, these chemicals do have certain use patterns in common. These inlcude use on apples, apricots, beans, cottonseed, certain citrus fruits, grapes, peaches, pears, pecans, plums, strawberries, and tomatoes.

VI. DATA GAP

The Agency does not know of any studies which tested for the oncogenicity of EPN. To insure a more complete evaluation of the risks posed by EPN, the Agency also requests registrants or any other person having information relevant to the possible oncogenicity of EPN (including preliminary reports in progress) to submit such data to the Agency.

EPN: Position Document 1

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* Copies of references not designated confidential will be provided on request. There will be a charge to cover duplicating costs for such requests. A copy of the Position Document and all references not designated confidential are available for public inspection in the Special Pesticide Review Division (TS-791), Office of Pesticide Programs, Envrionmental Protection Agency, Crystal Mall #2, Room 722, 401 M Street S.W., Washington, D.C. 20460.

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